Activation of Fas (CD95) in Adipocytes Contributes to High Fat Diet-Induced Insulin-Resistance

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Erklärung

Diese Dissertation wurde von PD Dr. Daniel Konrad betreut. Sie wurde selbständig und ohne unerlaubte Hilfe angefertigt. Bei der Abfassung der Dissertation wurden im Sinne der Promotionsordnung der Mathematisch-naturwissenschaftlichen Fakultät der Universität Zürich vom 8. Juli 2002 keine anderen als die angegebenen Hilfsmittel verwendet.

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1 SUMMARY

The prevalence of type 2 diabetes and insulin resistance has risen dramatically over the last decades. Excess energy intake resulting in obesity and dysregulation of white adipose tissue (WAT) are important factors contributing to the development of these metabolic diseases. The objective of this thesis was to investigate the role of adipocyte-expressed Fas (CD95) in the development of obesity-induced insulin resistance. In addition, insulin sensitivity of large and small adipocytes of obese white adipose tissue was investigated and compared.

Activation of the Fas signalling pathway in cultured 3T3-L1 adipocytes leads to increased secretion of pro-inflammatory cytokines like IL-6 and KC (murine analogue of IL-8). Moreover, Fas activation in 3T3-L1 adipocytes has negative effects on insulin sensitivity as shown by reduced insulin-stimulated glucose uptake. In vivo, Fas expression is increased in isolated adipocytes of insulin resistant mice and in adipose tissue of obese and diabetic patients. Adipocyte-specific Fas-knockout (AFasKO) mice were partly protected from HFD-induced deterioration of glucose homeostasis, and their levels of pro-inflammatory factors in WAT were reduced. Moreover, AFasKO mice did not develop hepatic steatosis and maintained hepatic insulin sensitivity. Thus, Fas activation in adipocytes contributes to adipose tissue inflammation, hepatic steatosis and insulin resistance induced by obesity.

Hypertrophic adipocytes, a consequence of obesity, are known to secrete higher amounts of pro-inflammatory factors compared to small adipocytes. Thus, we hypothesized that large adipocytes may be more insulin resistant. Interestingly, we found that insulin sensitivity did not differ

between small and large adipocytes isolated from the epididymal fat pad of obese mice. However, large adipocytes secreted higher amounts of free fatty acids (FFA) compared to small adipocytes under basal conditions.

In conclusion, “dysregulated” adipocytes in obese white adipose tissue contribute to the development of local and/or systemic insulin resistance. Large, lipid loaded adipocytes secrete higher amount of FFA, which might negatively affect insulin sensitivity. Moreover, the Fas (CD95) pathway is up-regulated in obesity and seems to contribute to local and systemic insulin resistance via changes in the secretion pattern of cytokines.

1 ZUSAMMENFASSUNG

Die Prävalenzen von Typ 2 Diabetes und Insulin Resistenz haben in den letzten Jahren massiv zugenommen. Übergewicht, verursacht durch einen Überschuss an Energiezufuhr, sowie eine Disregulierung des weissen Fettgewebes sind wichtige Faktoren, die zu diesen metabolischen Krankheiten beitragen. Das Ziel dieser Arbeit war es, den Einfluss einer Fas-Rezeptor (CD95)-Aktivierung in Fettzellen bei der Entstehung der übergewichts-bedingten Insulinresistenz zu untersuchen. Zusätzlich wurde die Insulinsensitivität von kleinen und grossen Fettzellen, welche aus dem gleichen Fettgewebe übergewichtiger Mäuse isoliert wurden, analysiert und verglichen.

Die Aktivierung des Fas-Signalweges in kultivierten Fettzellen (in vitro) führt zu einer vermehrten Sekretion von Entzündungsfaktoren wie IL-6 und KC (murines IL-8) aus diesen Zellen. Zudem vermindert die Aktivierung des Fas-Rezeptors die Insulinsensitivität von 3T3-L1 Adipozyten. In vivo ist die Expression des Fas-Rezeptors in Fettzellen insulinresistenter Mäuse sowie im Fettgewebe übergewichtiger und diabetischer Patienten erhöht. Mäuse, bei denen der Fas-Rezeptor spezifisch in Fettzellen ausgeschaltet wurde (AFasKO), sind teilweise vor einer Verschlechterung des Zuckerstoffwechsels (Glukosetoleranz), welche durch eine fettreiche Ernährung induziert wurde, geschützt. Zudem exprimiert das Fettgewebe dieser Mäuse weniger Entzündungsfaktoren. AFasKO Mäuse entwickeln, im Gegensatz zu Wildtyp-Mäusen, keine Fettleber bei fettreicher Ernährung. Auch die Insulinsensitivität der Leber bleibt erhalten. Folglich trägt die Aktivierung des Fas-Rezeptors in Fettzellen zur Übergewichts-

induzierten Entzündung des Fettgewebes, zur Entstehung der Fettleber sowie der Insulinresistenz bei.

Hypertrophe Fettzellen, welche sich vermehrt im Fettgewebe von Übergewichtigen finden, sezernieren mehr Entzündungsfaktoren als kleine Fettzellen. Wir stellten die Hypothese auf, dass hypertrophe Fettzellen vermehrt insulinresistent sind. Überraschenderweise fanden wir, dass die Insulinsensitivität zwischen kleinen und grossen Fettzellen, welche aus dem gleichem Fettgewebe übergewichtiger Mäuse isoliert wurden, gleich ist. Grosse Fettzellen sezernieren aber mehr freie Fettsäuren und tragen möglicherweise so zu einer vermehrten Ganzkörper-Insulinresistenz bei.

Zusammfassend lässt sich sagen, dass eine Disregulation des Fettzellstoffwechsels, wie sie im Rahmen einer Adipositas auftreten kann, eine wichtige Rolle in der Entstehung der lokalen und systemischen Insulinresistenz spielt. Grosse, lipidreiche Fettzellen sezernieren vermehrt freie Fettsäuren. Fettsäuren können die Insulinsensitivität sowohl lokal, im Fettgewebe, wie auch systemisch vermindern. Des Weiteren können Fettzellen ihr Sekretionsmuster aufgrund vermehrter Fas-Rezeptor-Aktivierung verändern. Eine vermehrte Freisetzung von Entzündungsfaktoren kann sowohl lokal wie auch systemisch (hepatisch) eine Insulinresistenz induzieren.

2 INTRODUCTION

2.1 Type 2 diabetes and insulin resistance

2.1.1 Type 2 diabetes

The prevalence of type 2 diabetes has risen dramatically over the last two decades. Moreover, it is anticipated that the worldwide incidence of this metabolic disease will increase by about 70% over the next 20 years. Type 2 diabetes can lead to blindness, end-stage renal disease, and non-traumatic loss of limbs. Besides generating enormous medical costs, type 2 diabetes is the 5th leading cause of death [1]. In addition to a genetic predisposition, acquired factors such as obesity and sedentary lifestyle play an important role in its pathogenesis. While type 1 diabetes mellitus is defined by a heterogeneous disorder characterized by β-cell loss [2], the onset of type 2 diabetes is most often preceded by insulin resistance, characterised by a reduced cellular sensitivity to insulin. During early stages of insulin resistance, pancreatic β-cells can compensate for attenuated cellular insulin response by increasing insulin production and secretion and, thus, raising insulin levels (hyperinsulinemia). However, type 2 diabetes finally develops as β-cells fail to compensate for peripheral insulin resistance [3].

2.1.2 Insulin

The discovery of the protein hormone insulin 1921 was one of the major medical advances of the last century. Insulin is synthesized and secreted by pancreatic β-cells in the islet of Langerhans. Insulin binding to the insulin receptor, a protein tyrosine kinase composed of two α- and two β-subunits, leads to its activation via autophosphorylation of the β-subunit.

Further downstream, cell and tissue specific signalling pathways can be activated and/or inhibited [4]. Insulin is a pleiotropic hormone with several effects on metabolism and body homeostasis. The major function of insulin is to lower circulating blood glucose levels, which is mainly mediated by insulin-stimulated glucose uptake into skeletal muscle (and to a minor part adipose tissue and even kidney) and by insulin-induced suppression of glucose release (gluconeogenesis and glycogenolysis) from the liver. Inhibition of free fatty acid (FFA) release from adipose tissue and stimulation of protein synthesis in skeletal muscle are other important metabolic functions of insulin [5].

2.1.3 Insulin signalling

Insulin-stimulated glucose uptake into adipose tissue and skeletal muscle is mediated by the facilitative glucose transporter GLUT4. Upon interaction of insulin with its receptor at the plasma membrane, the insulin receptor tyrosine kinase is activated, leading to phosphorylation of the insulin receptor substrate (IRS) family of proteins on several tyrosine residues. Tyrosine-phosphorylated IRS serve as docking sites for phospatidylinositol 3-kinase (PI3-K) (Fig. 1). Downstream of PI3-K, the serine/threonine protein kinase Akt (also called protein kinase B, PKB) as well as the atypical protein kinase C isoforms λ and ζ are activated and contribute to insulin regulated GLUT4 translocation to the plasma membrane [6]. Besides glucose uptake, insulin promotes glycolysis as well as glycogen and protein synthesis in skeletal muscle. In adipocytes, insulin decreases FFA release (lipolysis), via decreased activity of protein kinase A (PKA) and, further downstream, hormone sensitive lipase (HSL). Moreover,

insulin stimulates FFA uptake via activation of lipoprotein lipases (LPL) into adipocytes and increases the rate of intracellular lipid synthesis [7].

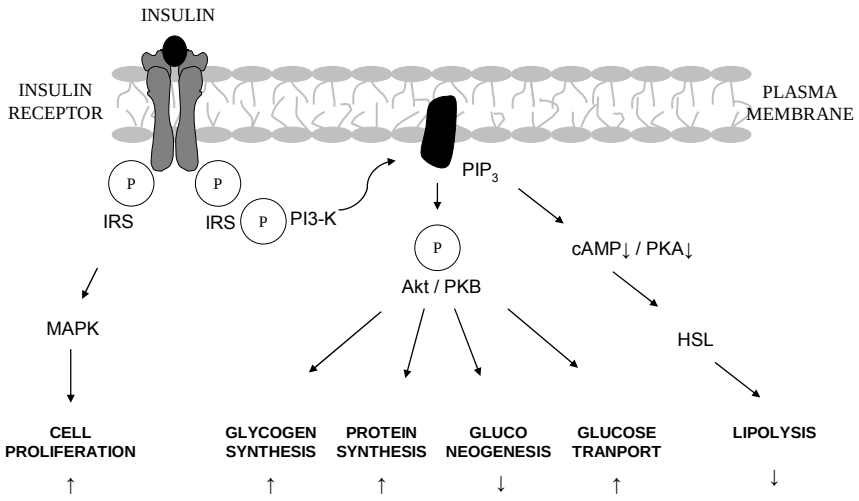

Fig. 1 Insulin signalling

Propagation of the insulin signal involves activation of PI3-K and of its downstream target Akt leading to increased glucose transport, elevated glycogen and protein synthesis and/or suppressed gluconeogenesis (liver). Moreover, lipolysis is inhibited in adipocytes via decreased activation of PKA. In addition, mitogenic pathways leading to cell proliferation can be induced via activation of mitogen activated protein kinases (MAPK). P: phosphorylation [6-9].

The liver plays an important role in the maintenance of blood glucose homeostasis. Although there are no insulin sensitive glucose transporters in the liver, insulin plays an important role in the regulation of hepatic glucose turnover. Insulin inhibits glucose output from the liver by down regulating the rate limiting enzymes of gluconeogenesis (phosphoenolypyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase)) and by decreasing the rate of glycogenolysis. At the same time, insulin increases hepatic lipid and glycogen synthesis, the latter via activation of glycogen synthase [8].

2.1.4 Insulin resistance

In insulin resistance, transmission of the insulin signal is reduced in one or several locations. In particular, phosphorylation of IRS on specific serine residues inhibits tyrosine phosphorylation of IRS by decreasing its affinity to the insulin receptor kinase, resulting in reduced transmission of the insulin signal. Several cytokines such as tumour necrosis factor-alpha (TNF-α) as well as FFAs were shown to activate serine/threonine kinases leading to phosphorylation of IRS on specific serine residues [10-12]. Numerous studies implicated activation of c-Jun N-terminal kinase (JNK) and/or increased production of nuclear factor-kappa B (NF-κB) with increased serine phosphorylation of IRS, and hence, with the induction of insulin resistance [13, 14]. Moreover, cytokine-induced expression of SOCS proteins (suppressors of cytokine signalling) was shown to inhibit insulin signalling by preventing binding of IRS to the tyrosine-phosphorylated insulin receptor [15]. Besides impaired insulin signalling at the level of IRS, there are other „critical nodes“ that can be affected in insulin resistance [9]. PI3-K generates phosphatidylinositol (3,4,5)-triphosphate (PIP_3), a lipid second messenger that is important for the activation of Akt/PKB further downstream (Fig. 1). Phosphatases such as phosphatase and tensin homologue (PTEN) can dephosphorylate and inactivate PIP_3 and therefore negatively affect insulin signalling. Moreover, the phosphorylation of Akt/PKB can be directly inhibited by several mechanisms [9].

Muscle, adipose and hepatic insulin resistance contribute all to the development of type 2 diabetes. Decreased insulin-stimulated glucose uptake by skeletal muscle and decreased suppression of hepatic glucose production (gluconeogenesis) are responsible for the excessive rise in

plasma glucose levels upon glucose ingestion, i.e. postprandial hyperglycemia (in the absence of a compensatory rise in insulin secretion). Moreover, insulin resistant adipocytes fail to respond to the anti-lipolytic actions of insulin, which leads to elevated plasma FFA. Chronically increased FFA levels may have deleterious effects on hepatic and skeletal muscle insulin sensitivity as well as on beta-cell function and therefore further worsen glucose homeostasis [16].

2.2 White adipose tissue and obesity

2.2.1 White adipose tissue (WAT)

Adipogenesis, the development of adipose tissue, includes the differentiation of preadipocytes of mesenchymal origin into mature adipocytes. During differentiation, specific transcription factors like proliferator-activator receptor γ (PPARγ) and members of the CCAATT enhancer-binding proteins (C/EBPs) are activated and lead to the expression of adipocyte-specific proteins like GLUT4 and fatty acid binding protein 4 (FABP4). Besides adipocytes, WAT consists of other cell types such as macrophages, fibroblasts, blood cells, endothelial cells and preadipocytes forming the stromal vascular fraction (SVF). This heterogeneity of the adipose tissue leads to an extensive paracrine crosstalk between the different cells. Moreover, factors secreted from the WAT, like cytokines or chemokines, can affect several cells and tissues of the entire body in an endocrine manner (Fig. 2) [17].

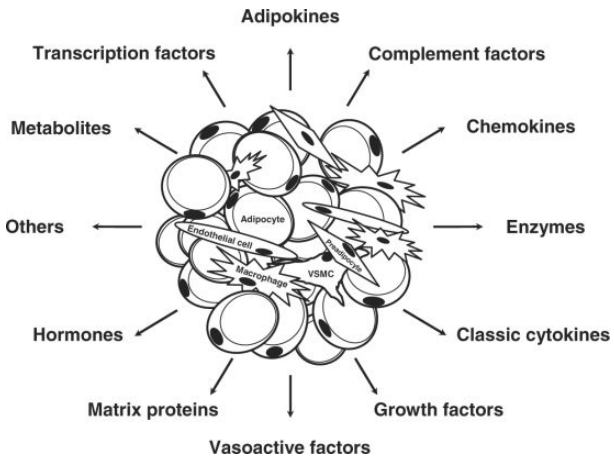

Fig. 2 Adipose derived factors

White adipose tissue (WAT) consists of several cells like adipocytes, macrophages and endothelial cells. Those cells secret different factors (adipokines, chemokines, metabolites e.g.) that act in a paracrine manner within the WAT, and in an endocrine manner affecting systemic homeostasis. Image adopted from [17].

Anatomically, adipose tissue depots can be divided in subcutaneous locations (approx. 80% of total body fat) and internal locations (approx. 20% of total body fat). In obesity, two main types of fat distribution can be distinguished: an “android obesity” type (“apple shaped”) with increased abdomino-visceral fat accumulation, and a “gynoid obesity” type (“pear shaped”) with increased subcutaneous fat [17].

2.2.2 Obesity

Obesity has reached epidemic proportions in Europe. The prevalence of obesity has increased approximately 30% over the past 10 to 15 years, rendering it an important factor for several diseases like type 2 diabetes and arteriosclerosis [18]. In particular, the number of overweight and obese children and adolescents has been increasing. In Switzerland, approximately every 5^{th} child is overweight [19]. Obesity is caused by caloric intake exceeding energy expenditure, and is associated with an increased number and/or size of white adipocytes storing the excessive energy in the form of triglycerides [20]. Under a prolonged period of positive energy balance, adipose tissue mass increases by enlargement of existing adipocytes (hypertrophy). Additionally, WAT expansion can progress by an increase in cell number (hyperplasia) [17]. Adipocyte hypertrophy was proposed to be causative in inducing adipose tissue dysfunction in obesity, since large adipocytes may secrete higher levels of pro-inflammatory cytokines [21]. On a more metabolic level, hypertrophic adipocytes may exhibit a reduced capacity to store and retain FFAs, leading to elevated levels of circulating FFAs [22].

In the U.S. more than 80% of patients with type 2 diabetes are overweight, pointing towards an important role of obesity in the development of type 2 diabetes [16]. Besides total body fat mass, the distribution of body fat seems to play an important role. Subjects with preferential upper body fat accumulation (“apple shaped”) are at higher risk for insulin resistance and type 2 diabetes than people with increased percentage of lower body fat accumulation (“peer shaped”). Probably, increased amounts of visceral adipose tissue in the “apple shaped” obesity contributes to this phenomenon, which leads to increased release of FFA and pro-inflammatory cytokines into the portal vein and the liver, respectively [16].

Importantly, WAT is needed for proper glucose homeostasis, as individuals with diminished fat mass (lipodystrophy) can develop insulin resistance. Insufficient adipose tissue mass in patients with lipodystrophy leads to an ectopic storage of FFA and triglycerides in other tissues (liver, skeletal muscle), and ectopic fat storage in skeletal muscle and liver correlates positively with insulin resistance in these tissues. These observations suggest that it is not merely the increase in adipose tissue mass per se that alters the function of target organs in obesity [16, 23], but also the location and “quality” of the white adipose tissue. Likewise, increasing intra-abdominal fat mass by transplantation of fat pads from non-obese mice had no negative effect on glucose homeostasis, but even increased insulin sensitivity [24]. This effect might be due to the fact that the transplanted intra-abdominal fat was stitched to the peritoneum and therefore was drained systemically via the vena cava and not by the portal vein.

2.2.3 White adipose tissue and insulin resistance

Adipose tissue plays an important role in the development/pathogenesis of insulin resistance. For many years, the sole function of white adipose tissue (WAT) was considered to be the storage of triglycerides. However, findings over the last two decades identified WAT as an important endocrine organ secreting different hormone-like factors (adipokines), FFAs as well as cytokines and thereby affecting metabolism locally and systemically [17, 25]. In obesity and insulin resistance, production of certain adipokines is reduced (e.g. adiponectin), whereas secretion of other adipokines/cytokines like IL-6 or TNF-α is increased [16]. This “dysfunctional” secretion pattern is characteristic for obese and/or diabetic subjects and may be related to increased fat accumulation. In this regard, hypertrophic adipocytes, a consequence of excess energy intake, are known to secrete increased levels of pro-inflammatory cytokines compared to small adipocytes [21].

Moreover, adipose tissue of obese subjects is infiltrated by macrophages and other inflammatory cells suggesting a role for inflammation in the pathogenesis of obesity and diabetes. The infiltrating inflammatory cells secrete different cytokines such as IL-1β, IL-6, IL-8 (KC), TNF-α and MCP-1, which in turn alter the expression and secretion pattern of adipokines and cytokines in adipose tissue. As a consequence, insulin sensitivity is impaired locally and systemically [11, 26, 27]. In mice, 3 days of high fat diet (HFD) is enough to induce a significant infiltration of intra-abdominal WAT by neutrophils, whereas longer periods of HFD leads to an accumulation of macrophages in WAT [28-33]. There are different types of macrophages in adipose tissue. Resident macrophages, which are

predominantly found in WAT of lean subjects, are thought to be alternatively activated and mainly producing anti-inflammatory cytokines like IL-10. Bone marrow-derived macrophages that infiltrate adipose tissue in obesity, however, are classically activated and secrete pro-inflammatory factors like TNFα and IL-6. Moreover, diet-induced obesity leads to a shift in polarization of resident, alternatively activated macrophages towards classically activated, pro-inflammatory macrophages [34].

Besides inflammatory processes induced by macrophages and other immune cells, insulin resistance in adipose tissue can also be evoked by endoplasmic reticulum stress (ER stress). FFAs were shown to induce ER stress in several cells by provoking the unfolded protein response (UPR). The UPR, a source of stress signalling, can activate JNK or promote oxidative stress, which are both known to induce insulin resistance [35].

2.2.4 Systemic cross-talk

Factors that are secreted by adipose tissue (Fig. 2) can affect other tissues and organs of the body. Importantly, skeletal muscle and the liver, two key components in the control of glucose homeostasis, may be affected, i.e. hepatic and muscular insulin sensitivity can be impaired by obesity-induced alterations in adipokine secretion and by elevated cytokine and FFA release (Fig. 3) [26, 36, 37].

Particularly, increased delivery of FFA from expanded adipose tissue to the liver can contribute to an excessive accumulation of hepatic triglycerides, which is characteristic for nonalcoholic fatty liver disease (NAFLD). In addition, cytokines seem to contribute to the pathogenesis of NAFLD [38]. Especially, IL-6 and TNF-α were suggested to play a

significant role in the development of fatty liver since they are up-regulated in patients with NAFLD [39-41].

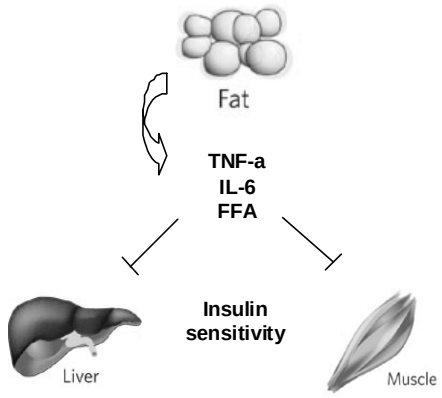

Fig. 3 Systemic cross-talk

In obesity, fat tissue secretes several factors that can affect glucose metabolism systemically. For example, TNF-α, IL-6 or FFA secreted by WAT can decrease insulin sensitivity in liver and skeletal muscle, therefore negatively affecting glucose homeostasis.

2.3 Fas (CD95)

2.3.1 Apoptosis

Fas (also known as CD95) is a member of the tumour-necrosis factor receptor superfamily and plays a central role in the regulation of programmed cell death (apoptosis) in many cell types. Fas is expressed in most tissues, and resistance to Fas receptor-mediated cell death is associated with the progression and metastasis of tumours. Activation of the Fas signalling pathway by binding of Fas ligand (FasL) to Fas initiates a caspase cascade that culminates in apoptosis. Trimerisation of the Fas receptor is necessary for the recruitment of Fas associated death domain (FADD) and formation of the death inducing signalling complex (DISC). Further downstream, caspases 8 (FLICE) and caspase 3 (the “effector caspase”) are activated and provoke apoptosis [42]. The Fas-mediated death signal can be inhibited by the FLICE-inhibitory protein (FLIP), which competes with caspase 8 for binding to FADD.

2.3.2 Non-apoptotic pathway

In addition to this well-established role in apoptosis, activation of Fas can also induce non-apoptotic signalling pathways including cell proliferation and inflammation [1, 43]. Importantly, Fas activation was shown to induce the secretion of cytokines such as IL-1α, IL-1β, IL-6, IL-8 (KC) and MCP-1 in different cell lines and tissues (Fig. 4) [44-48]. The signalling pathways involved in these Fas-mediated biological effects are only recently being unravelled. Activation of the extracellular signal-related kinase (ERK) [49], NF-κB [43, 50], or JNK [51-53] are possible mediators of the non-apoptotic Fas signalling pathway.

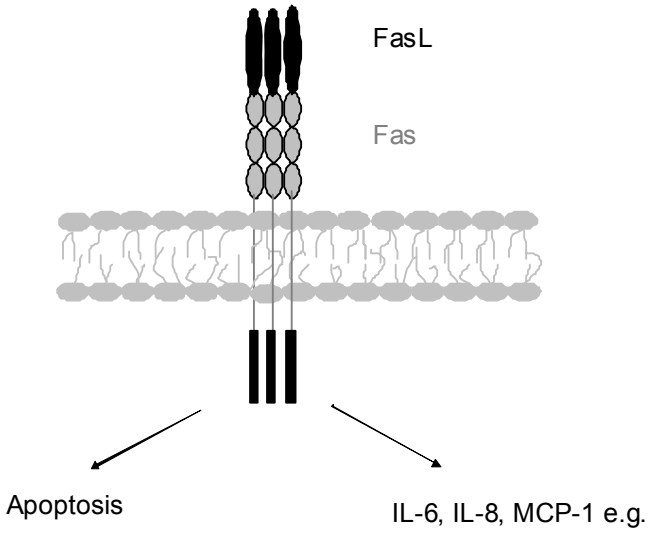

Fig. 4 Fas (CD95) signalling

Activation of the Fas signalling pathway by binding of Fas ligand (FasL) can lead to apoptosis via activation of caspases. However, non-apoptotic pathways leading to induction of cytokines like IL-6, IL-8 or MCP-1 can also be activated in several tissues and cell lines.

Factors modulating the balance between Fas-induced apoptotic and non-apoptotic pathways are not well known, but there is increasing evidence that FLIP may orchestrate this balance. FLIP appears to switch Fas activation from acting as a pro- to an non-apoptotic signalling event, leading to proliferation and/or inflammation [54, 55]. This effect of FLIP appears to depend on the strength of the Fas stimulus. At low FasL concentration, FLIP seems to be able to block apoptotic pathways, whereas at high doses this blocking effect is abolished, and apoptosis occurs [56]. In addition, death domain-associated protein (Daxx), which can be recruited to the Fas receptor signalling complex, may modulate the balance between Fas-induced apoptotic and non-apoptotic pathways by interacting with apoptosis signalling kinase 1 (ASK1) [42, 57]. Moreover, internalization of

the Fas receptor upon FasL binding seems to play a role for downstream signalling. Possibly, receptor mediated endocytosis is required for efficient activation of the caspase cascade leading to cell death, whereas it is not required for non-apoptotic pathways induced by Fas [58].

2.3.3 Fas and insulin resistance

As mentioned, Fas activation in several tissues and cells induces the secretion of different cytokines such as IL-1β, IL-6 and MCP-1. Intriguingly, several of theses pro-inflammatory cytokines are increasingly produced and secreted in obesity and insulin resistance. Thus, Fas activation may be a modulator of cytokine production in obesity and, thus, influencing obesity-induced insulin resistance. In addition, (non-apoptotic) Fas signaling can activate ERK, JNK and NF-κB, factors that are known to impair insulin sensitivity in several cells and tissues [13, 14].

Interestingly, recent studies in humans revealed an association of promoter alterations in the Fas and FasL gene with type 2 diabetes and insulin resistance [59], thus suggesting a potential role of these genes in the development of insulin resistance and/or type 2 diabetes.

2.4 Hypotheses

- Fas (CD95) activation in adipocytes contributes to obesity-induced insulin resistance
- Large adipocytes isolated from obese mice are more insulin resistant than small adipocytes harvested from the same fat pad

3 METHODS

3.1 Diverse methods

Methods used during the thesis are described in the Results section (Appendix; included manuscripts). Recently, the hyperinsulinaemic-euglycaemic clamp technique was established in our laboratory. This technique is described in detail below.

3.2 Hyperinsulinaemic-euglycaemic clamp

The hyperinsulinaemic-euglycaemic clamp technique is presently the "gold standard" for measuring insulin sensitivity in vivo. In this method, insulin is infused via a catheter intravenously to raise the insulin concentration to supraphysiological levels. At the same time, glucose is infused to maintain blood glucose at euglycaemic levels (5-6 mmol/l; "clamping" of blood glucose levels) [60].

Seven days before clamping, mice are anesthetized with isofluran and a catheter (MRE 025, Braintree Scientific, Braintree, MA, USA) is inserted into the left jugular vein and exteriorized at the back of the neck. Upon recovery, only mice that have regained > 90% of their preoperative weight are studied. After a fasting period of 5 hours, 3-[^{3}H]glucose (^{3}H-G) (0.1µCi/min; PerkinElmer, Schwerzenbach, Switzerland) is infused for 80 min and blood is collected from the tail tip for basal turnover calculation. After basal sampling, insulin (18 mU/kg x min) is infused for 90 min. Euglycaemia is maintained by periodically adjusting a variable infusion of 20% glucose with a syringe pump (TSE Systems, Bad Homburg, Germany). The glucose infusion rate is calculated as the mean of the steady state infusion which is reached after about 60 min of insulin and glucose infusion

(Fig. 4). A blood sample is collected after steady state infusion. The glucose turnover rate is calculated by dividing the rate of ^{3}H-G infusion by the plasma ^{3}H-G specific activity. Hepatic glucose production is calculated by subtracting the glucose infusion rate from the glucose turnover rate [61].

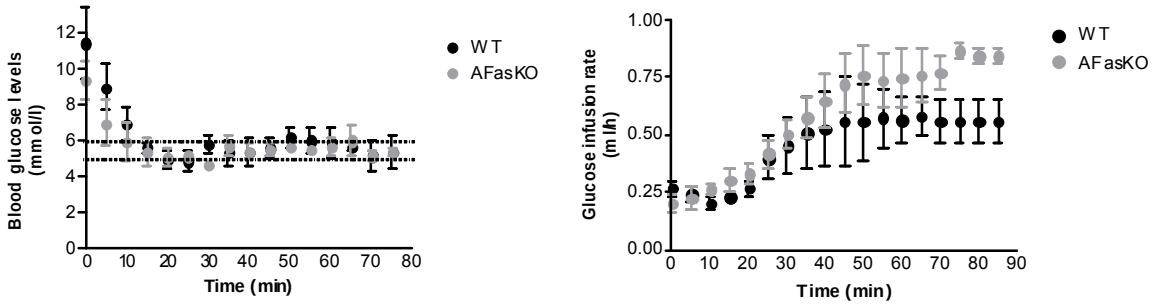

Fig. 4 Hyperinsulinaemic-euglycaemic clamp

After a prime infusion of 3-[^{3}H]glucose for 80 min, insulin and 3-[^{3}H]glucose are infused via catheter into the jugular vein (time point 0 min). Left graph: Blood glucose levels during a hyperinsulinaemic-euglycaemic clamp of WT and AFasKO mice (see attached manuscript). Blood glucose levels are “clamped” at 5-6 mmol/l (see dotted line). Right graph: To maintain euglycaemia, increasing amounts of glucose needs to be infused. Glucose infusion rates increase until a “steady state” infusion is reached after about 60 min. Steady state glucose infusion is calculated as the mean infusion rate between 60 and 90 min.

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5 RESULTS

5.1 Deletion of Fas (CD95) in adipocytes relieves adipose tissue inflammation and hepatic manifestations of obesity

Stephan Wueest, Reto A. Rapold, Desiree M. Schumann, Julia M. Rytka, Anita Schildknecht, Alexander V. Chervonsky, Assaf Rudich, Eugen J. Schoenle, Marc Y. Donath, Daniel Konrad. *Submitted*

5.2 Basal lipolysis, not the degree of insulin resistance, differentiates large from small isolated adipocytes in high-fat fed mice

Stephan Wueest, Reto A. Rapold, Julia M. Rytka, Eugen J. Schoenle, Daniel Konrad. *Diabetologia* published online

Publications are attached in the appendix.

6 DISCUSSION

White adipose tissue (WAT) plays an important role in the pathogenesis/development of insulin resistance. In obesity, excess storage of triglycerides leads to a “dysfunction” of adipocytes, leading to increased secretion of several adipocytokines (TNF-α, IL-1β, IL-6 and MCP-1 e.g.) and free fatty acids (FFA). Increased secretion of these pro-inflammatory cytokines and FFA can lead to local and/or systemic insulin resistance. The altered secretion pattern of these factors is mediated by inflammatory cells that infiltrate adipose tissue in obesity (e.g. macrophages), and by the modified function of (hypertrophic) adipocytes.

Although hypertrophic adipocytes isolated from obese subjects secrete more inflammatory cytokines compared to small adipocytes [21], we found no difference in insulin sensitivity between small and large adipocytes harvested from the same fat pad. However, large adipocytes have a higher rate of basal lipolysis, therefore releasing more FFA into the tissue and the circulation. Those results suggest that the degree of cellular hypertrophy is not a direct determinant of the severity of adipocyte insulin resistance. More likely, increased insulin resistance in adipocytes of HFD-fed mice might rather be the consequence of an increased release of pro-inflammatory cytokines and/or FFA of large adipocytes. Small and large adipocytes are affected in the same way, as both are exposed to the same “milieu”, and therefore show the same degree of insulin resistance. The reason for the increased release of FFA and pro-inflammatory cytokines from large and hypertrophic adipocytes needs to be further elucidated.

Fas (CD95), a member of the tumour necrosis-factor super family, is a cell membrane receptor that can trigger apoptosis. However, recent

findings in several cells like fibroblasts and pancreatic β-cells also point towards an inflammatory role of the Fas signalling pathway. Accordingly, activation of the Fas signalling pathway in adipocytes leads to increased expression of IL-6 and KC (IL-8), and therefore may contribute to inflammatory pathways that are activated in obesity. Increased expression of the Fas receptor in WAT of obese and diabetic subjects and in isolated adipocytes in mouse models of insulin resistance suggests a role for Fas activation in adipocytes in the pathogenesis of insulin resistance. Interestingly, Fas seems to be a target (it can be up-regulated in adipocytes by pro-inflammatory cytokines like TNF-α or IL-1β) and a positive mediator (it can increase secretion of pro-inflammatory cytokines from adipose tissue) of the low grade inflammation that occurs in obesity/insulin resistance. Adipocyte-specific Fas-knockout (AFasKO) mice showed increased adipocyte and hepatic insulin sensitivity compared to wildtype littermates, further suggesting a role for adipocyte-expressed Fas in obesity-induced insulin resistance.

In conclusion, excess energy intake leads to obesity and metabolic changes in adipose tissue. “Dysregulated” adipocytes in obesity secrete increased levels of pro-inflammatory factors, therefore contributing to the development of local and/or systemic insulin resistance. Large, lipid loaded adipocytes secrete higher amount of FFA, which can negatively affect insulin sensitivity. In addition, changes in secretion pattern of adipokines and cytokines, mediated e.g. by increased Fas activation in adipocytes, can lead to local and systemic insulin resistance. Hence, blocking the Fas signaling pathway in adipose tissue might be a promising target to reduce inflammatory processes induced by obesity. Moreover, reducing the

hypertrophic response of adipocytes to energy overload could reduce metabolic complications provoked by obesity.

7 APPENDIX

7.1 Abbreviations

AFasKO	Adipocytes specific Fas knockout
Akt	v-akt murine thymoma viral oncogene (PKB)
ASK1	Apoptosis signaling kinase 1
cAMP	Cyclic adenosine monophosphate
C/EBP	CCAATT enhancer-binding protein
CD95	Cluster of differentiation 95, Fas receptor
Daxx	Death domain-associated protein
DISC	Death inducing signalling complex
ER	Endoplasmic reticulum
ERK	Extracellular-signal related kinase
FABP4	Fatty acid binding protein 4
FADD	Fas-associated death domain
Fas-def	Total body Fas deficient
FasL	Fas ligand
FFA	Free fatty acid
FLIP	FLICE (caspase 8) inhibitory protein
G6Pase	Glucose-6-phosphatase
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
HFD	High fat diet
HSL	Hormone sensitive lipase
IL-10	Interleukin 10
IL-1α	Interleukin 1α
IL-1β	Interleukin 1β
IL-6	Interleukin 6
IL-8	Interleukin 8

ipGTT	Intra-peritoneal glucose tolerance test
ipITT	Intra-peritoneal insulin tolerance test
ipPTT	Intra-peritoneal pyruvate tolerance test
IRS	Insulin receptor substrate
JNK	C-Jun N-terminal kinase
KC	Mouse IL-8 analogue
LPL	Lipoprotein lipase
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemo attractant protein 1
mRNA	Messenger ribonucleic acid
NAFLD	Non-alcoholic fatty liver disease
NF-κB	Nuclear factor-kappa B
PEPCK	Phosphoenolypyruvate carboxykinase
PI3-K	Phosphatidylinositol 3-kinase
PIP_3	Phosphatidylinositol (3,4,5)-triphosphate
PKA	Protein kinase A
PKB	Protein kinase B
PPARγ	Proliferator-activator receptor γ
PTEN	Phosphatase and tensin homologue
Ser	Serine
SOCS	Suppressor of cytokine signalling
SVF	Stromal vascular fraction
TNF-α	Tumour necrosis factor-alpha
Tyr	Tyrosine
UPR	Unfolded protein response
WAT	White adipose tissue
WT	Wild-type

7.2 Acknowledgments

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7.4 Publications